IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

YU *et al.*

Appl. No. 09/314,889

Filed: May 19, 1999

For: **Death Domain Containing Receptors**

Art Unit: 1646

Examiner: Ulm, J.

Atty. Docket: 1488.0310006/EKS/SGW

Declaration for Deposited Biological Materials

Commissioner for Patents
Washington, D.C. 20231

Sir:

The undersigned attorney of record states as follows:

1. A plasmid containing human cDNA encoding DR3-V1 protein was deposited under the terms of the Budapest Treaty on October 10, 1996. The deposit was made at the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Virginia 20110-2209 (current address), and given accession number 97757. A copy of the ATCC deposit receipt reciting the term of the deposit, as well as the statement of viability, is attached hereto.

2. Assurance is hereby given that all restrictions on the availability to the public of the deposited plasmid referred to above will be irrevocably removed upon the granting of a patent issuing from the patent application captioned above.

DECEMBER 4, 2000
Date

Jonathan L. Klein
Attorney for Applicant
Registration No. 41,119

Human Genome Sciences, Inc.
9410 Key West Avenue
Rockville, MD 20850
Telephone: (301) 251-6015

031-6.DEC.DEPOSIT.WPD

American Type Culture Collection

12301 Parklawn Drive o Rockville, MD 20852 USA o Telephone: (301)231-5520 Telex: 898-055 ATCCNORTH o FAX: 301-770-2587

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF
THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

MAR 13 1996
HGS PATENT DEPT.

To: (Name and Address of Depositor or Attorney)

Human Genome Sciences, Inc.
Attn: Robert H. Benson
9410 Key West Avenue
Rockville, MD 20850

Deposited on Behalf of: Human Genome Sciences, Inc.

Identification Reference by Depositor:	ATCC Designation
DNA Plasmid, 231556	97456

The deposits were accompanied by: __ a scientific description _ a proposed taxonomic description indicated above.

The deposits were received March 1, 1996 by this International Depository Authority and have been accepted.

AT YOUR REQUEST:

X We will inform you of requests for the strains for 30 years.

The strains will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strains, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strains.

If the cultures should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace them with living cultures of the same.

The strains will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the cultures cited above was tested March 7, 1996. On that date, the cultures were viable.

International Depository Authority: American Type Culture Collection, Rockville, Md. 20852 USA

Signature of person having authority to represent ATCC:

Barbara M. Hailey

Barbara M. Hailey, Administrator, Patent Depository

Date: March 8, 1996

BP4/9

American Type Culture Collection

12301 Parklawn Drive ◇ Rockville, MD 20852 USA ◇ Telephone: (301)231-5520 Telex: 908-768 ATCCROVE ◇ FAX: 301-816-4366

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF
THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

Human Genome Sciences, Inc.
Attn: Robert H. Benson
9410 Key West Avenue
Rockville, MD 20850

Deposited on Behalf of: Human Genome Sciences, Inc.

Identification Reference by Depositor:	**ATCC Designation**
DNA Plasmid, 1198782 (Docket PF267P1.SKB)	97757

The deposits were accompanied by: __ a scientific description _a proposed taxonomic description indicated above.

The deposits were received October 10, 1996 **by this International Depository Authority and have been accepted.**

AT YOUR REQUEST:

X We will inform you of requests for the strains for 30 years.

The strains will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strains, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strains.

If the cultures should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace them with living cultures of the same.

The strains will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the cultures cited above was tested October 17, 1996. **On that date, the cultures were viable.**

International Depository Authority: American Type Culture Collection, Rockville, Md. 20852 USA

Signature of person having authority to represent ATCC:

Barbara M. Hailey

Date: October 19, 1996

Barbara M. Hailey, Administrator, Patent Depository

BH4/9

CURRENT PROTOCOLS IN MOLECULAR BIOLOGY

VOLUME 1

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IN SITU HYBRIDIZATION AND IMMUNOHISTOCHEMISTRY

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continued

SDS electrophoresis buffer, 5×

15.1 g Tris base
72.0 g glycine
5.0 g SDS
H_2O to 1000 ml
Dilute to 1× or 2× for working solution, as appropriate

Do not adjust the pH of the stock solution, as the solution is pH 8.3 when diluted. Store at 0° to 4°C until use (up to 1 month).

SED (standard enzyme diluent)

20 mM Tris·Cl, pH 7.5
500 µg/ml bovine serum albumin (Pentax Fraction V)
10 mM 2-mercaptoethanol
Store up to 1 month at 4°C

Sodium acetate, 3 M

Dissolve 408 g sodium acetate·$3H_2O$ in 800 ml H_2O
Add H_2O to 1 liter
Adjust pH to 4.8 or 5.2 (as desired) with 3 M acetic acid

Sodium acetate buffer, 0.1 M

Solution A: 11.55 ml glacial acetic acid/liter (0.2 M).
Solution B: 27.2 g sodium acetate ($NaC_2H_3O_2·3H_2O$)/liter (0.2 M).

Referring to Table A.2.2 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H_2O to 100 ml. (See Potassium acetate buffer recipe for further details.)

Sodium phosphate buffer, 0.1 M

Solution A: 27.6 g $NaH_2PO_4·H_2O$ per liter (0.2 M).
Solution B: 53.65 g $Na_2HPO_4·7H_2O$ per liter (0.2 M).

Referring to Table A.2.3 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H_2O to 200 ml. (See Potassium phosphate buffer recipe for further details.)

SSC (sodium chloride/sodium citrate), 20×

3 M NaCl (175 g/liter)
0.3 M Na_3citrate·$2H_2O$ (88 g/liter)
Adjust pH to 7.0 with 1 M HCl

STE buffer

10 mM Tris·Cl, pH 7.5
10 mM NaCl
1 mM EDTA, pH 8.0

TAE (Tris/acetate/EDTA) electrophoresis buffer

50× stock solution:	*Working solution, pH ~8.5:*
242 g Tris base	
57.1 ml glacial acetic acid	40 mM Tris·acetate
37.2 g $Na_2EDTA·2H_2O$	2 mM $Na_2EDTA·2H_2O$
H_2O to 1 liter	

TBE (Tris/borate/EDTA) electrophoresis buffer

10× stock solution, 1 liter:
108 g Tris base (890 mM)
55 g boric acid (890 mM)
40 ml 0.5 M EDTA, pH 8.0 (20 mM)

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IN SITU HYBRIDIZATION AND IMMUNOHISTOCHEMISTRY

14

continued

1c. *Harsh treatment:* Pour several hundred milliliters of boiling 0.1% SDS onto the membrane. Cool to room temperature.

If a membrane is to be reprobed, it must not be allowed to dry out between hybridization and stripping. If it becomes dry, the probe may bind to the matrix.

2. Place membrane on a sheet of dry Whatman 3MM filter paper and blot excess liquid with a second sheet. Wrap the membrane in plastic wrap and set up an autoradiograph.

If signal is still seen after autoradiography, rewash using harsher conditions.

3. The membrane can now be rehybridized. Alternatively, it can be dried and stored for later use.

Membranes can be stored dry between Whatman 3MM paper for several months at room temperature. For long-term storage, place the membranes in a desiccator at room temperature or 4°C.

REAGENTS AND SOLUTIONS

Aqueous prehybridization/hybridization (APH) solution

5× SSC (*APPENDIX 2*)
5× Denhardt solution (*APPENDIX 2*)
1% (w/v) SDS

Add 100 µg/ml denatured salmon sperm DNA (see below) just before use

Alternatives to Denhardt solution and denatured salmon sperm DNA as blocking agents are listed in Table 2.10.5 (see discussion in critical parameters).

Denatured salmon sperm DNA

Dissolve 10 mg Sigma type III salmon sperm DNA (sodium salt) in 1 ml water. Pass vigorously through a 17-G needle 20 times to shear the DNA. Place in a boiling water bath for 10 min, then chill. Use immediately or store at –20°C in small aliquots. If stored, reheat to 100°C for 5 min and chill on ice immediately before using.

Formamide prehybridization/hybridization (FPH) solution

5× SSC (*APPENDIX 2*)
5× Denhardt solution (*APPENDIX 2*)
50% (w/v) formamide
1% (w/v) SDS

Add 100 µg/ml denatured salmon sperm DNA (see above) just before use

Alternatives to Denhardt solution and denatured salmon sperm DNA as blocking agents are listed in Table 2.10.5 (see discussion in critical parameters).

Commercial formamide is usually satisfactory for use. If the liquid has a yellow color, deionize as follows: add 5 g of mixed-bed ion-exchange resin [e.g., Bio-Rad AG 501-X8 or 501-X8(D) resins] per 100 ml formamide, stir at room temperature for 1 hr, and filter through Whatman no. 1 paper.

CAUTION: *Formamide is a teratogen. Handle with care.*

Labeling buffer

200 mM Tris·Cl, pH 7.5
30 mM $MgCl_2$
10 mM spermidine

Mild stripping solution

5 mM Tris·Cl, pH 8.0
2 mM EDTA
0.1× Denhardt solution (*APPENDIX 2*)